CHROMOSOME NUMBER OF *LAUBERTIA CONTORTA* (APOCYNACEAE: APOCYNOIDEAE) AND ITS PHYLOGENETIC IMPORTANCE

Justin K. Williams and Dawn P. Derr[1]

Department of Biological Sciences
Sam Houston State University
Huntsville, Texas 77341-2116, U.S.A.

ABSTRACT

The mitotic chromosome count for *Laubertia contorta* (2*n* = 18) is the first reported chromosome count for the genus. A discussion of the relationship of *Laubertia* to other genera and the use of chromosome numbers in constructing non-molecular phylogenies in the Apocynaceae is presented. A distribution map and color photo of the species is also provided.

KEY WORDS: *Laubertia*, Apocynaceae, chromosome, *Forsteronia*, *Prestonia*, Mexico

RESUMEN

Se realiza el recuento de cromosomas en mitosis de *Laubertia contorta* (2*n* = 18) que es el primero el género. Se presenta una discusión de la relación de *Laubertia* con otros géneros y el uso de los números cromosomáticos en la construcción de filogenias no-moleculares en Apocynaceae. Se aporta también un mapa de distribución y fotografía en color de la especie.

A cursory review of chromosome numbers in the Apocynaceae (Van der Laan & Arends 1985; Goldblatt & Johnson 2003) indicates that 73 of the 179 genera in the Apocynaceae have been counted. Few chromosome counts in the Apocynaceae are meiotic because plants characteristically produce only between 3-8 flowers per inflorescence. In addition, because the anthers produce little pollen, it is difficult to fix the anthers at the proper meiotic phase for counting. Therefore most counts in the Apocynaceae are mitotic counts made from the region of cell division in root tips using the "squash" technique (Witkus 1951; Raffauf 1964). The majority of taxa counted are those commonly cultivated and therefore with readily accessible root tips. Van der Laan and Arends (1985) discussed in detail the systematic utility of chromosome numbers in the Apocynaceae. Based on their observations, Van der Laan and Arends (ibid) suggested that chromosome numbers have the potential for resolving some important relationships in the Apocynaceae. In addition, they pointed out that only eight of the 55 genera in the Americas have had chromosome counts reported, and they strongly suggested that more counts be pursued.

Laubertia A. DC. comprises three species of Neotropical lianas native from Central Mexico to northern South America (Morales 2002). One species, *Laubertia contorta* (Mart. & Gal.) Woodson, is endemic to Mexico, where it ranges from Sinaloa to Chiapas (Fig. 1) and is readily distinguished from the other species of the genus by its twisted corolla tube and deep maroon corollas lobes (Fig. 2a). The relationship of *Laubertia* is disputed (Williams 1999), with some authors relating it to *Echites* P. Browne and others to *Prestonia* R. Br. (Morales 2002). A morphological cladistic analysis (Fig. 3, Williams 2004) places *Laubertia* basal to a clade of four species of *Prestonia* (here referred to as the "*Prestonia*" clade). Although *Laubertia* was nested with *Prestonia*, the bootstrap support for this relationship was below 50%. Chromosome numbers were not used for one of the character states in this analysis. Since the time of this analysis chromosome counts in the Apocynaceae have accumulated, including the one presented here. This paper provides the first chromosome count for *Laubertia* and examines the utility of chromosome numbers in morphological cladistic analyses.

MATERIALS AND METHODS

Voucher and fruiting specimens (Fig. 2b) were collected during January 2003 in Mexico (Fig. 1) by the first author. Voucher specimens were mounted and deposited at SHST and GH. MEXICO. CHIAPAS: 25 ft inside

[1]**Current address: 14 Twin Feather Place, The Woodlands, Texas 77381, U.S.A.**

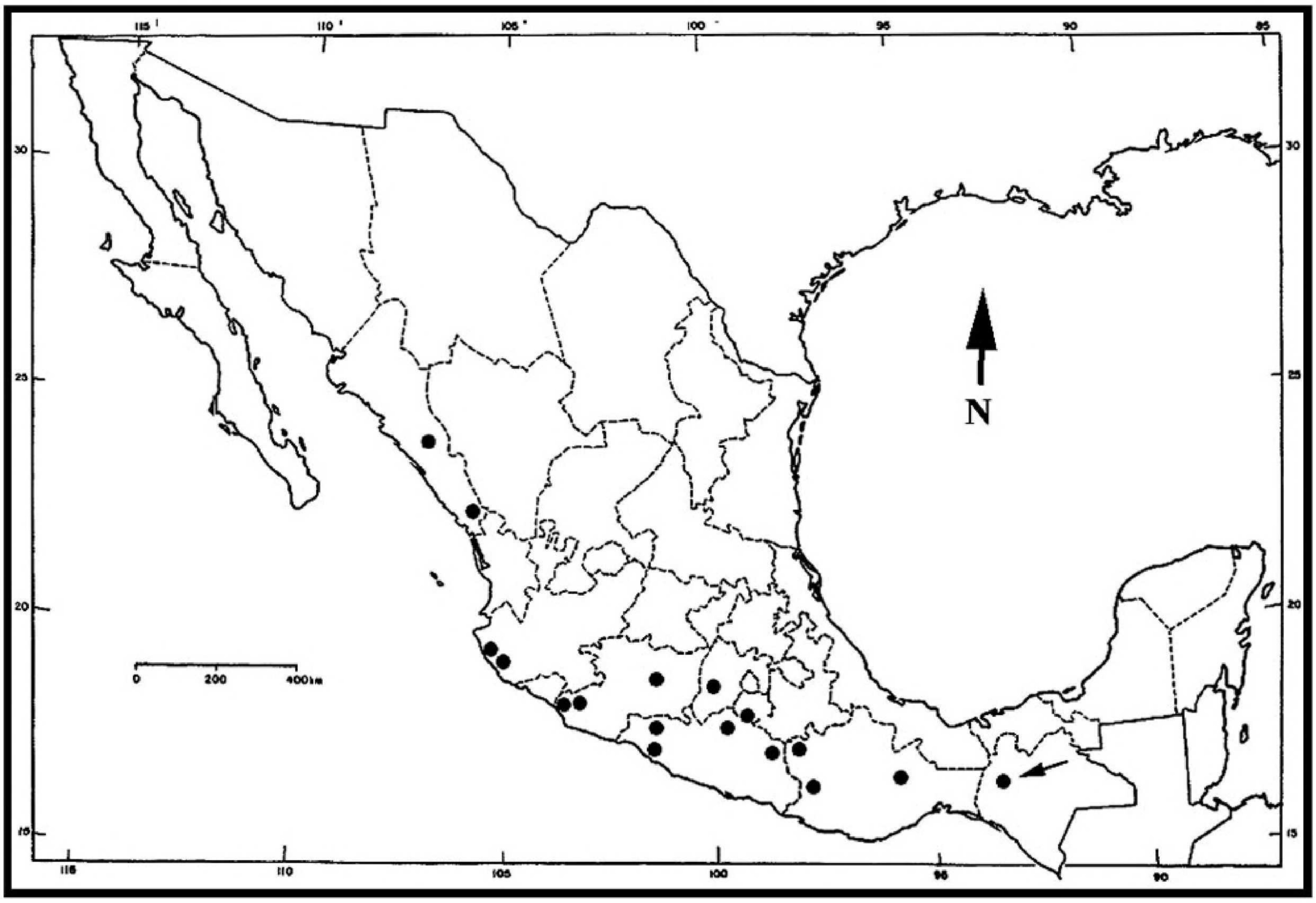

Fig. 1. Distribution of *Laubertia contora* (from Williams 1999). The arrow points to the site where follicles were collected.

the park from the entrance to "El Choreoderro" waterfall park ca. 10 mi N of Tuxtla Gutiérrez, 2 Jan 2003, *Williams 2003-1* (SHST, GH).

The collected seeds (Fig. 2c) were planted in small starter pots with commercial potting soil in July 2003 and were maintained in the greenhouse at the Department of Biological Sciences at Sam Houston State University. Twenty-four seeds were planted with no additional measures performed, such as soaking or scarification. Fifty percent of the seeds germinated (Fig. 2d) over a three month period. Once the plants developed several leaves (Fig. 2e) and an extensive root system, actively growing root tips (Fig. 2f) were collected, fixed, and analyzed using standard procedures (Van der Laan & Arends 1985).

Finally, using the same taxa, character matrix, and methodology in Williams (2004), we re-analyzed the data adding an additional character: chromosome number. Chromosome numbers for 29 of the 45 taxa included in Williams (ibid) were identified from the present study and a literature search (Van der Laan & Arends 1985; Goldblatt & Johnson 2003). The genera identified with chromosome counts were then scored based on their respective base number: 0: $x = 11$ (*Adenium* Roem. & Schult., *Apocynum* L., *Nerium* L.); 1: $x = 10$ (*Cerbera* L., *Strophanthus* DC., *Thevetia* L.); 2: $x = 9$ (*Forsteronia* G. Mey, *Laubertia*, *Parsonsia* R. Br., *Prestonia*); 3: $x = 6$ (*Echites*, *Odontadenia* Benth., *Pentalinon* Voigt); 4: $x = 8$ (*Mandevilla* Lindl.).

RESULTS

A mitotic chromosome number of $2n = 18$ or a base number of $x = 9$ was observed for *Laubertia contorta*. Van der Laan and Arends (1985) reported chromosome length in the Apocynaceae between 0.5–4 µm, with the average chromosome length between 1–2 µm. The length of the chromosomes in *L. contorta* varies between 0.5–1.5 µm, consistent with other chromosomes in the Apocynaceae.

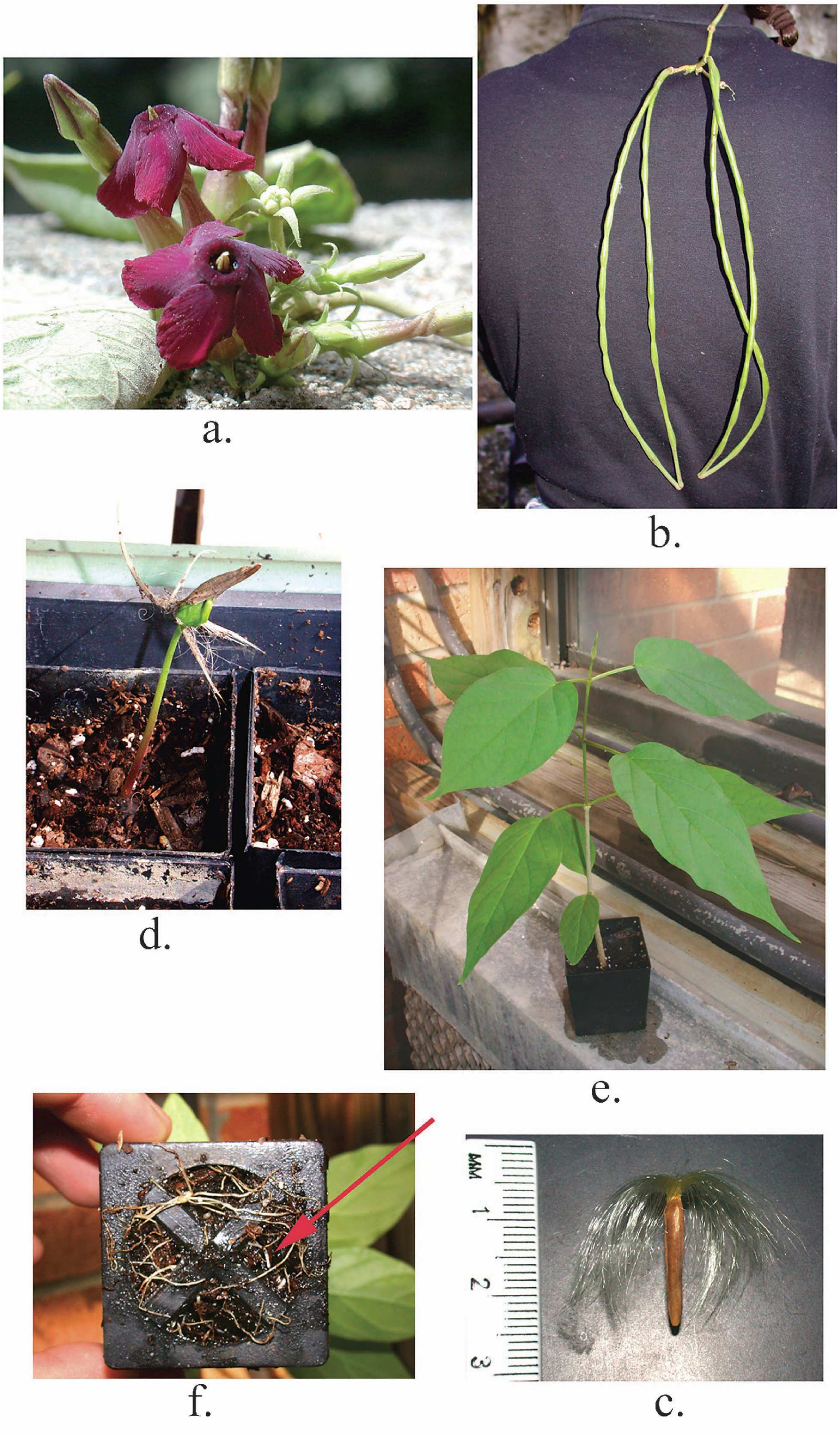

Fig. 2. Photographs of various stages and parts of *Laubertia contorta* **a.** Corolla; **b.** follicles; **c.** Seed with coma; **d.** emerging seedling; **e.** juvenile plant with leaves; **f.** root tips.

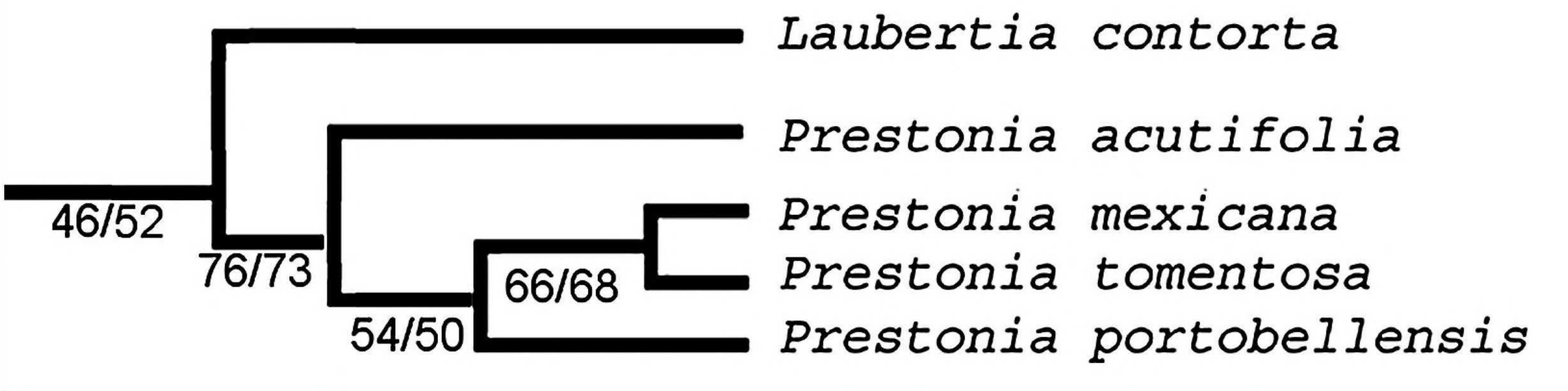

Fig. 3. Dendogram of *"Prestonia"* clade (from Williams 2004). Numbers below branches indicate bootstrap values. The first number indicates values determined in this study and the second number is from the analysis presented in Williams 2004. Only the value for the *"Laubertia/Prestonia"* branch determined in this analysis is included as the previous value was below 50%.

The heuristic search in Williams (2004) yielded a total of 48 equally parsimonious trees of 159 steps. The new search with chromosome counts added yielded a total of 98 equally parsimonious trees of 164 steps. The bootstrap value for the *Prestonia* clade (Fig. 3) was below 50% (46%) when chromosomes were not included (Williams 2004) and over 50% (52%) when chromosomes were included.

DISCUSSION

Van der Laan and Arends (1985) reported $x = 11$ as the base chromosome number for the Apocynaceae based on its prevalence in the family and on the observation that many of the plesiomorphic taxa possess a base number of $x = 11$. A review of the chromosomes numbers in the Apocynaceae presented by Van der Laan and Arends (ibid) indicates that a base chromosome number of $x = 9$ is found in eight genera represented in four of the 21 tribes recognized in the Apocynaceae (Endress & Bruyns 2000): Mesechites (*Forsteronia*), Echiteae (*Laubertia*, *Parsonsia*, *Prestonia*), Malourtieae (*Pachypodium* Lindl.), and Plumerieae (*Allamanda* L, *Plumeria* L.). The Plumerieae is in the subfamily Rauvolfioideae (anthers free from pistil head and aestivation of corolla bud sinistrorse) and differs from the other three tribes which are in the Apocynoideae (anthers fused to the pistil head and aestivation of corolla bud dextrorse). Based on subfamilial and tribal circumscription (Endress & Bruyns 2000) along with phylogenetic evidence (Williams 2004) it is suggested here that the evolution of $x = 9$ evolved independently in potentially four different clades within the Apocynaceae.

A cursory review of chromosome counts of the Apocynaceae (Van der Laan & Arends 1985; Goldblatt & Johnson 2003) shows that at present the only chromosome counts for Apocynoideae genera suggested and potentially related to *Laubertia* are for *Echites* ($x = 6$), *Forsteronia* ($x = 9$), *Parsonsia* ($x = 9$), *Pentalinon* ($x = 6$), and *Prestonia* ($x = 9$). The increased bootstrap value for the *Laubertia/Prestonia* (Fig. 3) clade calculated with the additional character state (chromosome numbers) provides further support for the close relationship between the two genera. And although the re-sampling of the data matrix, only increased the bootstrap value of the *Laubertia/Prestonia* by 6%, this increased the value of the clade to over 50%, which is often utilized as the lower threshold value for the beginning of support in phylogenies constructed from large scale data-sets (Sanderson & Wojciechowski 2000) like this one. In addition, the results indicate that chromosome numbers are useful in constructing phylogenies in the Apocynaceae. Based on the phylogenetic significance of chromosome counts as potential tribal synapomorphies, it is suggested that further attempts be made to secure chromosome counts for other neotropical genera of the Apocynoidae, specifically *Allotoonia* Morales & J.K. Williams, *Angadenia* Miers, *Fernaldia* Woodson, *Rhabdadenia* Muell-Arg., *Mesechites* Muell-Arg., *Thenardia* H.B.K., *Thoreauea* J.K. Williams and additional species of *Echites* and *Prestonia*.

ACKNOWLEDGMENTS

We thank Tami Cook for providing us with access to her digital light microscope, Andrew Dewees for verifying the different mitotic stages and the chromosome count, Guy Nesom and an anonymous reviewer for making valuable edits to the manuscript and Bob Rhodes for mixing the Carnoy's solution and aceto-orcein. We also thank Jon Plum for providing photographs of *Laubertia contorta* flowers (Fig. 2a).

REFERENCES

ENDRESS, M.E. and P. BRUYNS. 2000. A revised classification of the Apocynaceae s.l. Bot. Rev. 66:1–56.

GOLDBLATT, P. and D.E. JOHNSON. 2003. Index to plant chromosome numbers 1998–2000. Monogr. Syst. Bot. Missouri Bot. Gard. 94.

MORALES, J.F. 2002. Studies in Neotropical Apocynaceae I: A revision of the genus *Laubertia*. Rhodora 104: 170–185.

RAFFAUF, R.F. 1964. Some chemotaxonomic considerations in the Apocynaceae. Lloydia 27:288–298.

SANDERSON, M.J. and M.F. WOJCIECHOWSKI. 2000. Improved bootstrap confidence limits in large-scale phylogenies, with an example from neo-*Astragalus* (Leguminosae). Syst. Biol. 49:671–685.

VAN DER LAAN, F.N. and J.C. ARENDS. 1985. Cytotaxonomy of the Apocynaceae. Genetica 68:3–35.

WILLIAMS, J.K. 1999. A phylogenetic and taxonomic study of the Apocynaceae subfamily Apocynoideae of Mexico with synopsis of subfamily Plumerioideae. Ph.D. dissertation. Austin: The University of Texas, Austin.

WILLIAMS, J.K. 2004. Polyphyly of the genus *Echites* (Apocynaceae: Apocynoideae: Echiteae): evidence based on a morphological cladistic analysis. Sida 21:117–131.

WITKUS, E.R. 1951. The chromosome number of several species of *Strophanthus*. Bull. Torrey Bot. Club 78: 80–82.